Sugar beet root growth under different watering regimes: a minirhizotron study

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Abstract

The yield of sugar beet is often reduced by drought stress and it has previously been shown that water uptake, especially from deeper layers of the soil profile, may be limited by inadequate total root length. Experiments were conducted to assess root growth at different depths in response to specific watering regimes. Sugar beet plants were grown in wooden boxes (2.16 m^2 x 1.2 m) in a polytunnel in two consecutive years. Minirhizotrons allowed regular monitoring of root growth at five different depths. Only when water in the upper soil layers had been depleted, did roots start proliferating in deeper soil layers. This development of the root system architecture, together with a lag between roots arriving at depth and actively taking up water, led to a delay in water being extracted from those deeper layers. During the period when roots were proliferating at depth, stomatal conductance reduced, indicating that the plants were suffering from water stress despite there still being water available. Even though new soil layers with high water availability were explored the stomatal conductance did not recover.

Keywords: Water uptake, drought, stomatal conductance, root plasticity

1. Introduction

Worldwide, water availability is an increasing problem for crops due to climate change. In addition to increasing average temperatures which will lead to higher water demand, there are likely to be more weather extremes resulting in periods with high water influx alternated with periods of drought (Rosenzweig et al. 2001; Kumar 2016; Kurnik and Hildén 2017). These dry periods can cause severe problems during critical stages of crop growth with a lower yield as a result (Araus et al. 2002; Ober and Luterbacher 2002; Pathan et al. 2014).

Sugar beet (*Beta vulgaris*) is grown in temperate regions all over the world and makes up 20% of the sugar production in the world, sugar cane providing the other 80% (FAO Investment Centre Division 2009). In the UK, sugar beet are mostly grown in East Anglia, where the soil type is predominantly sandy loam with an available water capacity of around 0.14 m^3 m^{-3} (Qi et al. 2005). Additionally, East Anglia is one of the drier regions in the UK with average annual rainfall being <600 mm in the past 10 years (MetOffice 2018). As a result, there is an average 10% yield loss due to low water availability which can exceed 25% in dry years (Jaggard et al. 1998).

Low water availability is not the only limitation to water uptake. Other factors that play a role are compaction and root tissue development. Compaction results in poor root growth, often at depth, and this, in turn, results in reduced water uptake from compacted soil layers (Kirkegaard and Lilley 2007). Root tissue development can be limiting when new roots are initially formed and the xylem tissue has not matured for optimum water uptake, as reported in grapevine and sugar beet (Mapfumo et al. 1993; Fitters et al. 2017).

Roots are known to have high plasticity and this allows them to adjust to environmental changes (York et al. 2016). Sugar beet root architecture is normally conical with many roots at shallow depths and a decrease in root length with increasing depth (Brown and Biscoe 1985). During periods of drought, roots proliferate in soil layers with higher water availability (Li et al. 2002; Padilla et al. 2013). In sugar beet, roots can grow to over one metre deep and take up water from that depth if there are no soil constraints (Fitters et al. 2017). However, when there is compaction, sugar beet hardly show any root proliferation in deeper layers before mild to severe drought occurs (Romano et al. 2012). Once drought occurs root proliferation at depth starts (Koevoets et al. 2016), but delays in root tissue development at that time can prevent immediate water uptake (Fitters et al. 2017).

Minirhizotrons have often been employed to look at root development over time (Johnson et al. 2001). Transparent tubes are placed in the soil and a special camera is inserted into the tube to take images of the roots growing against the tube. The advantages of this method are that it is non-destructive and allows multiple measurements over time (Jose et al. 2001). Some disadvantages of measuring root length with minirhizotrons are an underestimation of root lengths depending on the measurement depth, and preferential root growth along the tube (Parker et al. 1991).

Several studies have looked into root growth in sugar beet (Brown and Biscoe 1985; Brown et al. 1987), but over the past 30 years there have only been a few studies that focused on root growth in sugar beet which involved minirhizotrons. These studies were all done in field settings and the measuring depth varied from 0.7m to 2 m depth. These studies focussed mainly on root response differences between tillage methods, nitrogen fertilizer (van Noordwijk et al. 1994; Vamerali et al. 1999), and very little was done on responses to varying water availability (Vamerali et al. 2009). Studies that look at sugar beet root growth with minirhizotrons in controlled conditions are relatively rare, but necessary to get a better understanding about root growth under non-restricting conditions. Controlled minirhizotron studies can help answer questions concerning changes in root growth and how these changes might affect the overall plant development and health.

To fill in any existing knowledge gaps, a minirhizotron experiment was done in controlled conditions. This study aims to answer the following questions: a) How do sugar beet roots proliferate over time at different depths under differing water regimes?; b) How does the timing of drought affect root growth and plant development? To answer these questions two experiments were conducted over two years. In the first year question a) was addressed by assessing well-watered vs drought conditions. In year two, early drought vs late drought were compared, addressing question b).

2. Material and methods

2.1 Experimental design

Sugar beet were grown in six wooden boxes of 1.8 m x 1.2 m x 1.2 m (l x w x h) in 2016 and 2017. The soil medium was a sandy loam texture with an available PK content of 61 mg l^{-1} P and 850 mg l^{-1} K. and the boxes were emptied and filled with new soil between the two years. Assessment of penetration resistance showed that no compaction had occurred during filling, the resistance up to 75 cm was approximately 550 kPa. The boxes were arranged in a randomized block design with three blocks and were located in a polytunnel to exclude rainfall. The temperature fluctuated between -1 °C and 44 °C, with an average day temperature of 20 °C and an average night temperature of 11 °C. The boxes were filled in stages to encourage consolidation by watering at each stage before adding more soil. This was done several times until the boxes were filled to the top. Each box had four volumetric soil moisture sensors, EC-5 (Decagon Devices, Labcell Ltd., Alton Hants, United Kingdom) fitted at four depths: 20, 50, 80 and 110 cm. Five Em5b data loggers (Decagon Devices, Labcell Ltd., Alton, Hants, United Kingdom) were used to log the half hourly readings from the soil moisture sensors. Solid fertilizer (Nitram; CF® fertilisers, Billingham, Cleveland, USA) equivalent to 120 kg ha^{-1} (34.5% N) was applied on top of the soil as per field recommendation, no additional P and K was added. Each box contained ten horizontal minirhizotrons across the width of the box, two at each of the following depths: 30 cm, 50 cm, 70 cm, 90 cm, and 110 cm. To prevent over or under estimations the tubes were never placed in the same vertical plane. Prior to the start of the experiment, field capacity (25% volumetric soil moisture content) was determined by watering the boxes to saturation and then letting them drain for two weeks. The boxes were watered daily by trickle tape to maintain field capacity until the different watering regimes were imposed.

2.2 Drought response experiment (2016)

Three sugar beet seeds (cv. Haydn) were planted at 3 cm depth at each plant position, three rows of eleven plants. At c.25 DAS (Days After Sowing) the boxes were thinned to one seedling per position. The two watering regimes were: 1. continuous irrigation (control), boxes were watered on demand depending on the temperature and rate of water uptake to maintain soil moisture levels around 0.35 m^3 m^{-3}. 2. drought from 57 DAS onward (DR) (BBCH growth stage 1.16). Exact amounts of water given can be found in the supplementary table.

The youngest fully expanded leaf was used for weekly stomatal conductance measurements (mol m^{-2} s^{-1}) using an AP4 Porometer (Delta-T Devices, Burwell, Cambridge, United Kingdom)(Parkinson 1985). All measurements were taken between 9.00 h and 13.30 h. Roots in the middle 50 cm were imaged fortnightly through the minirhizotrons. The images (600 DPI) were taken at 1 cm intervals and then stitched together with ImageJ (Schindelin et al. 2012). The roots were traced manually after which the image was converted into a black and white format in ImageJ with the threshold color function (B&W) (Schindelin et al. 2012). WinRHIZO (Regent instruments Inc., Québec, Canada) was used to determine the root length. Relative leaf water content (RWC) was measured at 83 DAS and 126 DAS by measuring the fresh weight, turgid weight and dry weight of leaf discs taken from the plants (Turner 1981). At 131 DAS the DR plants were strongly suffering from drought and therefore the experiment was terminated and the plants were harvested. Leaf and root fresh weight and dry weight were determined, after drying at 75 °C for at least seven days. Total plant water use efficiency (WUE) was calculated from the total plant dry weight divided by the total water uptake during the whole experiment.

2.3 Drought timing experiment (2017)

Three sugar beet seeds (cv. Haydn) were planted at 3 cm depth at each plant location, three rows of seven plants. At c.25 DAS the boxes were thinned to one seedling per position. The watering regimes were: no irrigation between 60 – 145 DAS 'early drought' (EDR) (start at BBCH growth stage 1.15), and no irrigation between 128 – 178 DAS 'late drought' (LDR) (start at BBCH growth stage 4.44). When re-watering, small amounts of water (equivalent to 15 mm of water per day) were given at first to avoid surface run-off. Exact amounts of water given can be found in the supplementary table.

Stomatal conductance and root images were taken as described for 2016. Additional measurements were weekly SPAD measurements taken using a SPAD 502 plus meter (Konica Minolta, Tokyo, Japan). Between 132 DAS and 159 DAS the canopy temperature was recorded five times. A FLIR

148 thermal camera (FLIR® Systems Inc., Wilsonville, Oregon, USA), was used alongside the software
149 provided with the camera to assess the canopy temperature. Relative leaf water content (RWC) was
150 calculated at 76, 102 and 124 DAS.
151
152 At 215 DAS the plants were harvested after both treatments had had a chance to replenish. Leaf and
153 root fresh weight and dry weight of five beet in the middle of the front row were determined, after
154 drying at 75 °C for at least seven days. Total plant water use efficiency (WUE) was calculated from
155 the total plant dry weight and the total water uptake during the whole experiment. Six storage
156 roots, taken from the middle plants from each box were sent to the sugar factory to determine sugar
157 yield.
158
159 *2.4 Statistical analysis*
160 A general ANOVA for a randomized block design was performed on plant biomass data, sugar yield
161 data, and RWC measurements. For stomatal conductance, root length, soil moisture, canopy
162 temperature, and SPAD data, a repeated measures ANOVA was performed. GenStat 15th edition
163 (VSN International Ltd., Hemel Hempstead, United Kingdom) was used for the statistical analyses.
164
165
166 **3. Results**
167
168 *3.1 2016 – Drought experiment*
169 Under well-watered conditions there was considerable fluctuation in soil volumetric moisture
170 content (Fig 1a). These fluctuations were caused by watering events and plants taking up water.
171 However, the soil volumetric moisture content was kept above 0.25 m^3 m^{-3} for the majority of the
172 experiment. When irrigation was halted at 57 DAS, there was a slow decline in soil moisture content
173 at each depth (Fig 1b). Water was taken up at 20 cm immediately and, 8 days after drought started,
174 the soil moisture content started to reduce at 50 cm as well. From 80 DAS substantial moisture
175 reduction was also observed at 80 and 110 cm. Drought had strong impacts on water use efficiency
176 (WUE); the WUE of the DR plants was 8.4 g l^{-1} compared to 6.7 g l^{-1} in the control plants.

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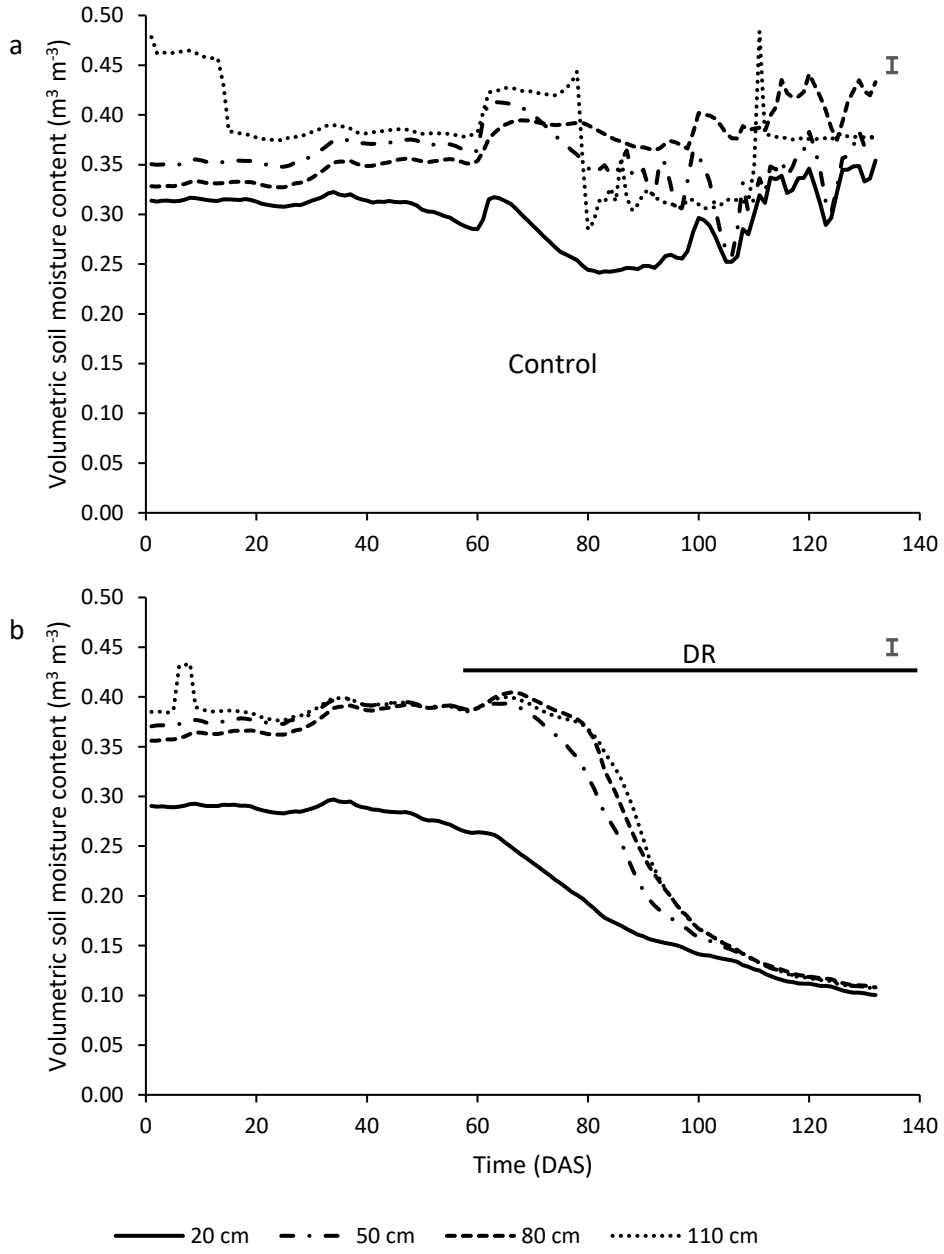

Figure 1 Volumetric soil moisture content (m^3 m^{-3}) over time at four different depths; 20, 50, 80 and 110 cm in 2016. Where a) Control and b) DR (drought). The solid horizontal bar shows the duration of the drought treatment. The error bar shows the least significant difference (time*treatment).

178

179 Over time, there were clear changes in root length and distinct differences between the treatments
180 (Fig 2). From 92 DAS there were significant differences in root length as a result of root proliferation
181 at 110 cm in the DR treated plants ($p < 0.001$, DF = 48, l.s.d. = 161.8) (Fig 2). These differences
182 persisted until the end of the experiment. The increase in roots at 110 cm coincided with reductions
183 in soil moisture at the corresponding time.

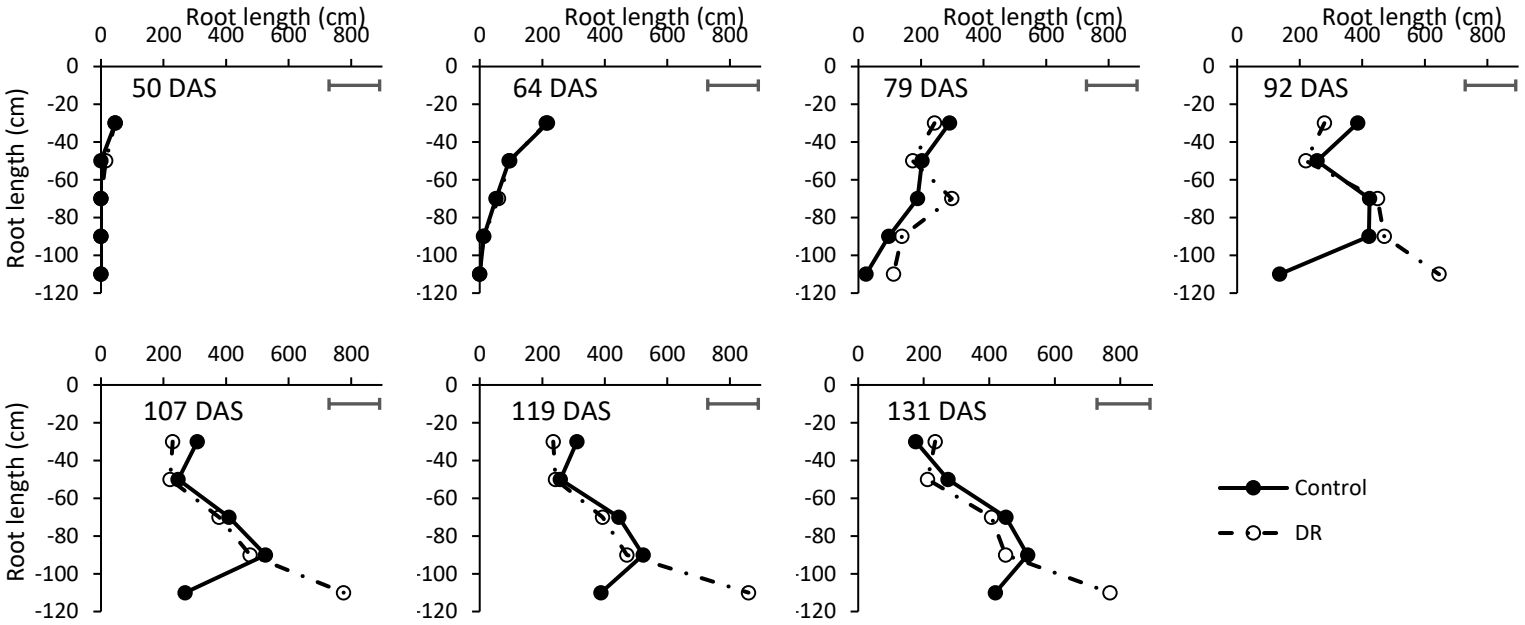

Figure 2 Root length (cm) at five different depths at consecutive time points in 2016. The error bar shows the least significant difference (time*treatment*depth).

184

185 From 97 DAS, 40 days after irrigation stopped for the DR treated plants, the control plants had a

186 higher stomatal conductance that the DR plants (p = 0.005, DF = 10, l.s.d. = 0.272) (Fig 3). At

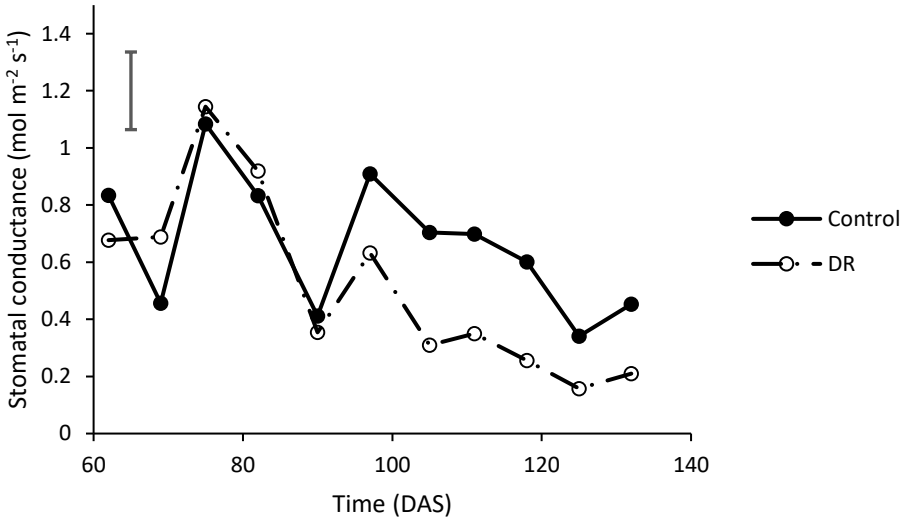

Figure 3 Stomatal conductance (mol m^{-2} s^{-1}) drought had started at the time of the first measurement in 2016. DR=drought treatment. The error bar shows the least significant difference (time*treatment)

187 approximately the same time (92 DAS), roots started to proliferate at 110cm depth (Fig 2).

188

189 At the end of the experiment there were large differences in dry weights. In the control treatment

190 the plants had a significantly higher overall dry weight of 217 g per plant against 124 g per plant in

the DR treatment ($p<0.001$; DF=1; lsd=36.57). A leaf dry weight of 107 g ($p<0.001$; DF=1; lsd=14.03) and root dry weight of 110 g ($p=0.04$; DF=1; lsd=49.5) compared to 67 g and 57 g in the DR plants respectively. The final root water content was three times as much in the roots of the control plants as in the DR treated plants and the leaf water content was 3.8 times as high in the control plants.

Relative leaf water content (RWC) measured at 83 DAS did not show any significant differences despite the DR plants not receiving any water for 26 days. At 127 DAS the DR treated plants had a significantly lower RWC ($p=0.026$; DF=1; lsd=9.83), 76% compared to 89% in the control plants. At this time the actual water content of the control plants was 92% and that of the DR treated plants was 85% ($p=0.016$; DF=1; lsd=0.364).

3.2 2017 – Drought timing

When watering stopped, in both the EDR and LDR treatments, there was a slow decline in moisture content at each depth (Fig 4a-b), with water being taken up from the top layers before being depleted from the bottom layers. When watering restarted there was no immediate increase in volumetric soil moisture content: it took approximately 30 days before replenishment was seen. After re-watering for 70 days the soil moisture content of the EDR treated plants had replenished to 91% of the starting volumetric water content (Fig 4a). The LDR plants were re-watered for 37 days, after which the soil moisture content was replenished to 78% of the starting value (Fig 4b). No differences in WUE were found between the different treatments.

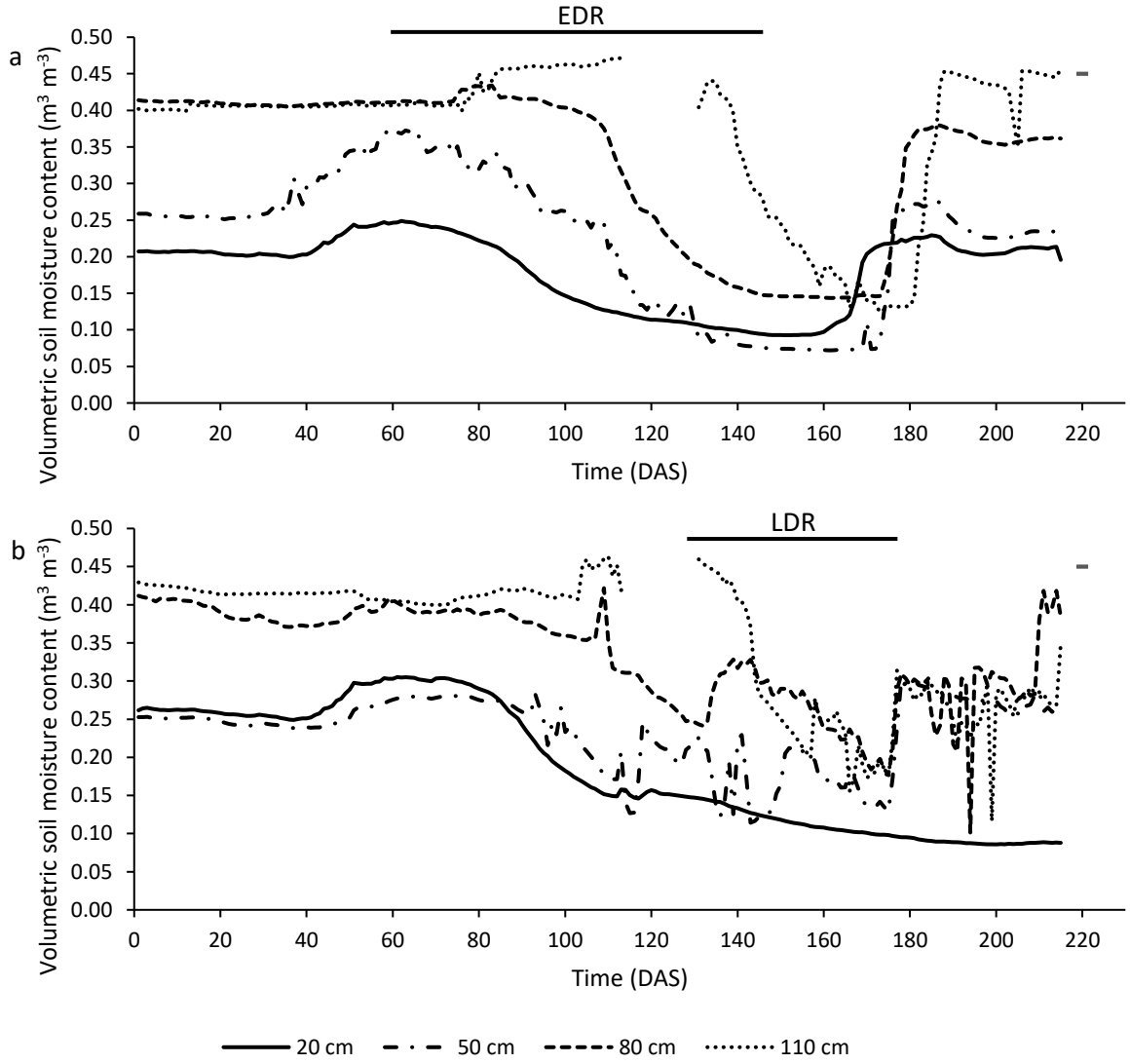

Figure 4 Volumetric soil moisture content (m^3 m^{-3}) over time at four different depths: 20, 50, 80 and 110 cm in 2017. a) the EDR treated boxes, b) the LDR treated boxes. The solid horizontal bars show the timing of early (EDR) and late (LDR) drought. The error bar shows the least significant difference. Due to sensor failure there was a loss of data at 110 cm depth in both treatments between 115 and 130 DAS. The error bar shows the least significant difference (time*treatement).

212 The first differences in root length appeared at 101 DAS (Fig 5), when the EDR treated plants had a
213 significantly greater root length at 70 cm, 41 days after drought started. Eleven days later the LDR
214 plants had caught up and the differences disappeared (Fig 5). At 90 cm a similar trend was seen
215 between 127 and 133 DAS, where the EDR plants had started proliferating at this depth first and
216 after 20 days the LDR plants had caught up (Fig 5). At 140 DAS most differences were found at 110
217 cm depth (Fig 5). However, when irrigation of the LDR plants stopped at 128 DAS, root proliferation

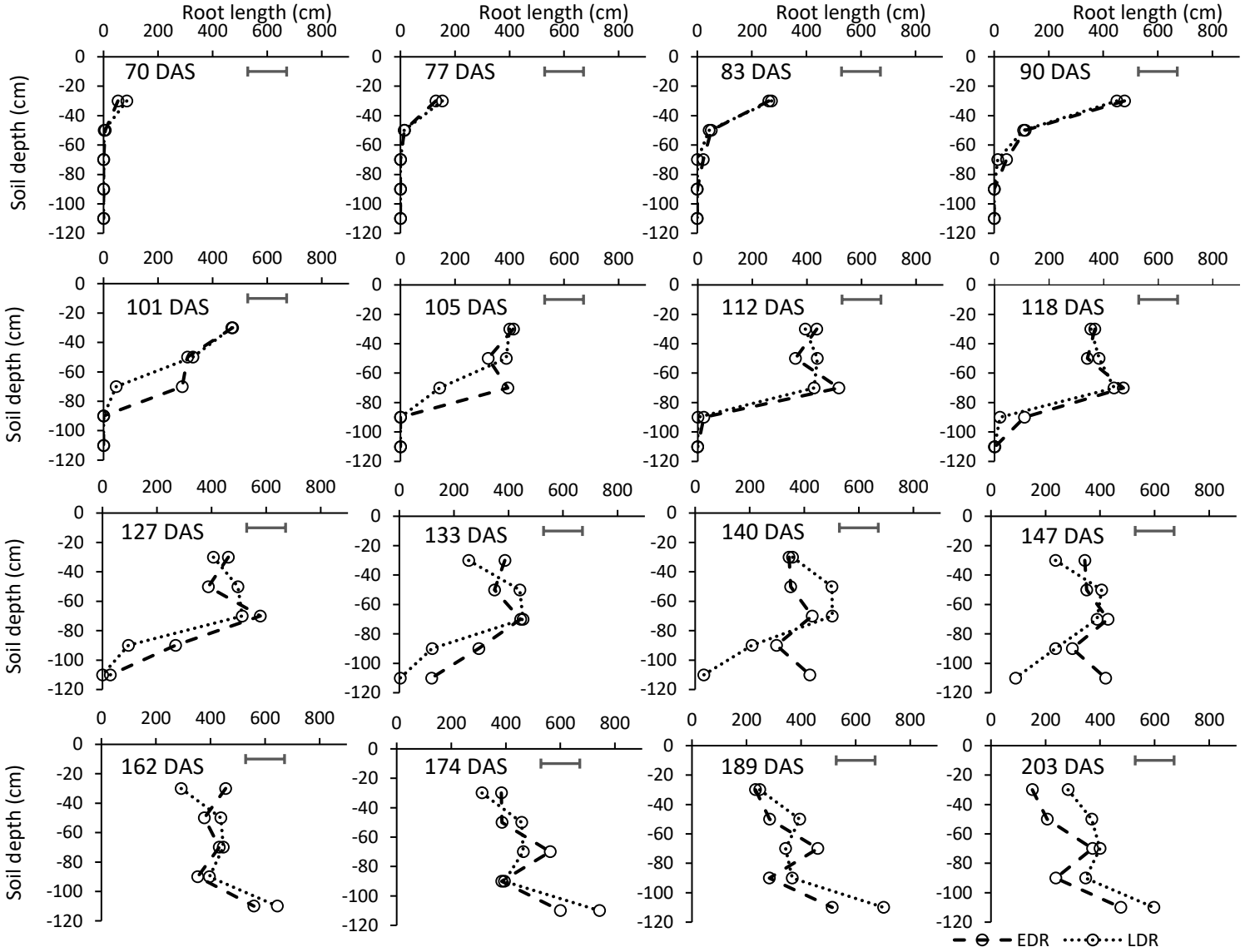

Figure 5 Root length (cm) at five different depths at successive time points in 2017. EDR=early drought, LDR=late drought. The error bar shows the least significant difference (time*treatment*depth)

218 at 110 cm depth was seen, resulting in the difference between treatments disappearing (Fig 5). At
219 203 DAS the LDR treated plants had a greater root length overall (Fig 5).
220

221 The first significant differences in stomatal conductance were observed at 110 DAS which coincided
222 with a decrease in soil moisture content at 80 cm in the EDR treated plants (Fig 6). At 145 DAS the
223 EDR plants were re-watered and from then there was an increase in stomatal conductance. From the
224 moment the irrigation of the LDR treated plants stopped at 128 DAS the stomatal conductance

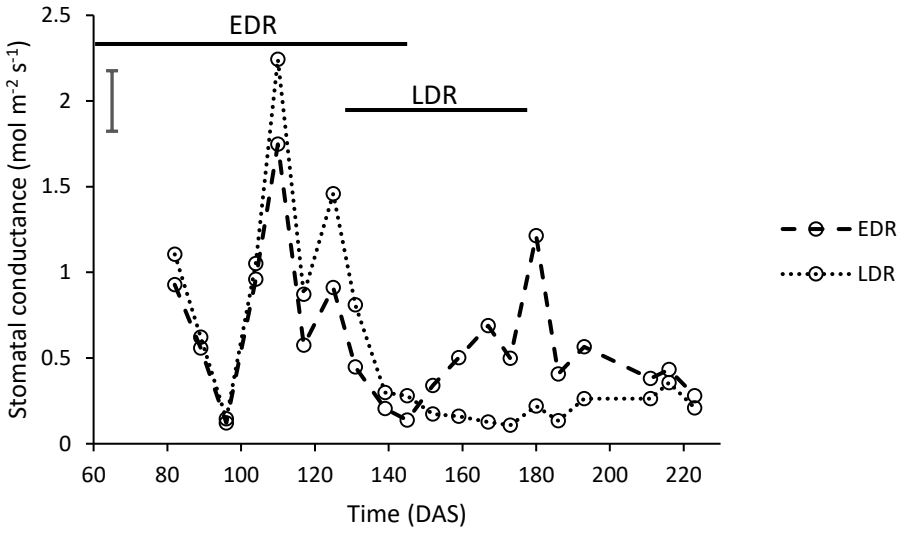

Figure 6 Stomatal conductance (mol m^{-2} s^{-1}) over time in 2017 for each treatment: EDR and LDR. The solid horizontal bars show the timing of early (EDR) and late (LDR) drought. The error bar shows the least significant difference.

225 became more like the EDR treated plants. After rewatering of the LDR there was again a slight
226 increase in stomatal conductance and at 186 DAS there were no more significant differences
227 between the two treatments.
228

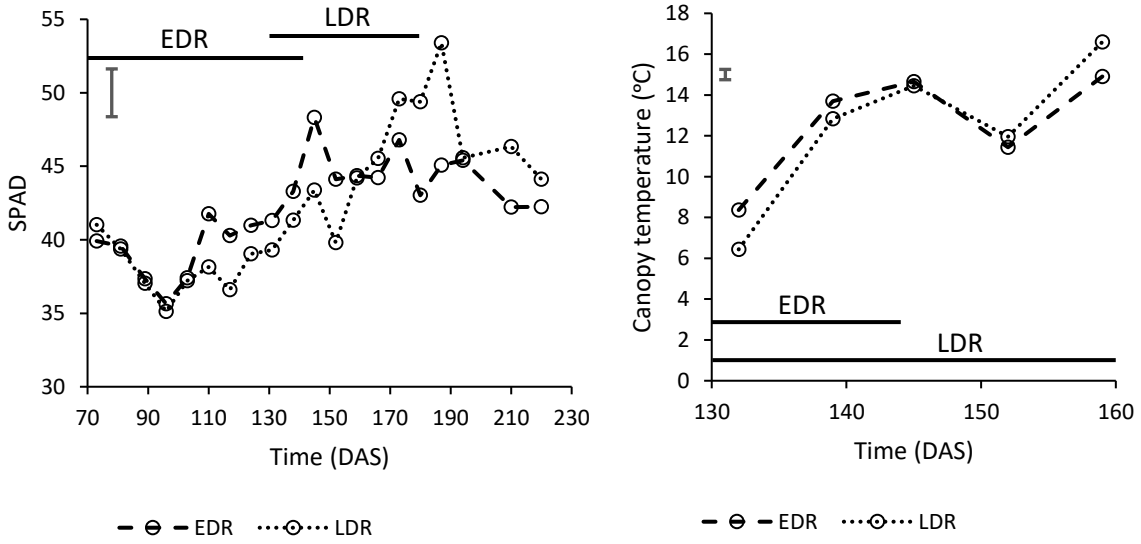

Figure 7 a) SPAD over time in 2017 for each treatment: EDR and LDR; b) Canopy temperature (°C) from 132 DAS to 159 DAS. The moment that the EDR treated plant have replenished and the LDR treated plants are starting to suffer from drought. The solid horizontal bars show the timing of early (EDR) and late (LDR) drought. The error bars show the least significant difference.

Weekly SPAD measurements showed no differences in SPAD until 110 DAS when the EDR treated plants had a significantly higher SPAD than the LDR treated plants (Fig 7a), likely caused by a concentration effect as a result of reduced water content. At 159 DAS the LDR treated plants' SPAD values went up as a result of drought stress while the EDR treated plants' SPAD values stabilised and even declined. After re-watering the SPAD values of the LDR plants declined again.

At the end of the experiment there were no significant differences in total dry weight or root dry weight. However, the EDR plants had a significantly higher leaf dry weight ($p=0.005$; DF=1; lsd=43.26), with the EDR plants having 278 g and the LDR plants only 139 g of leaf DW. There were no significant differences in RWC until 124 DAS, when the EDR treated plants had a RWC of 80% ($p<0.001$; DF=1; lsd=0.323), compared to 77% in the LDR plants. When the sugar content was determined at the end of the experiment it showed no significant differences. Water use efficiency (WUE) showed there were no significant differences between the two treatments, WUE was based on final root dry weight. EDR treated plants had a WUE of 0.7 g l^{-1}, compared to 0.8 g l^{-1} in the LDR treated plants.

Around the time the EDR plants were re-watered, regular canopy temperature measurements were taken (Fig 7b). When re-watering occurred, the canopy temperature dropped significantly below that of the LDR treated plants which were at that point subjected to drought.

4. Discussion

To increase water uptake under drying soil conditions, root proliferation at depth has often been observed in crops (Asch et al. 2005; Comas et al. 2013; Lobet et al. 2014). Our findings show that in sugar beet grown under both prolonged and short periods of drought, more deep roots were developed compared to sugar beet grown under fully irrigated conditions. In 2016, the soil moisture decreased rapidly from the top of the profile which resulted in roots mostly being formed at depth. In some layers a delay was observed between roots reaching a certain depth and water uptake from that depth. A possible explanation for this could be that the xylem had yet to mature before optimum water uptake could take place (Mapfumo et al. 1993; Fitters et al. 2017). In 2017, this delay between roots reaching a depth and there being water uptake was more pronounced. It is likely that the higher root imaging frequency in 2017 resulted in there being earlier detection of roots at each

depth and therefore a more precise estimation of when roots reached a given depth could be made. Differing water availability had a strong effect on the rooting patterns, especially at 110 cm depth. In both years most differences were found at 110 cm, with drought stressed plants showing strong root proliferation at this depth after approximately 20 days of drought. Similar rooting patterns under drought conditions were seen in other crops such as maize, wheat and sunflower (Wasson et al. 2012; Lynch 2013; Lilley and Kirkegaard 2016). The use of minirhizotrons in this study allowed close monitoring of root growth at the different depths (Johnson et al. 2001). As a result, differences caused by water limitations were seen in root growth before above ground measurements gave an indication of water being limited. Root proliferation at 110 cm depth under drought conditions was the most striking finding.

A decrease in water availability does not always result in an immediately quantifiable plant response (Pang et al. 2011). When drought occurred, stomatal conductance remained unchanged for at least 39 days in the case of the 2016 plants and the LDR plants in 2017. The EDR plants took longer with 50 days, possibly because the plants were smaller at that stage and therefore they did not use as much water. Another possible reason could be that the water demand was not as high since these plants were smaller when drought started compared to the LDR plants and the plants in 2016. However, when the drought persisted, stomata were closed which resulted in lower stomatal conductance at the cost of reduced photosynthesis. This is often seen as a plant protection mechanism to preserve water (Ober et al. 2005; Rivero et al. 2009). However, a previous study in soybean observed that the reduction in photosynthesis was not immediate (Daryanto et al. 2016). Daryanto et al. (2016) found that at first, photosynthesis decreased less rapidly compared to stomatal conductance. This might be since several photosynthetic processes are influenced by stomatal conductance and not the other way around (Medrano et al. 2002). Stomatal conductance was regulated in the short term by the plant evaporative demand. The long term regulation was led by conditions of water extraction from the soil (Tardieu and Davies 1993).

Stomatal conductance is often used as an indication of drought stress. Flexas and Medrano (2002) stated that a stomatal conductance below 0.1 mol m^{-2} s^{-1} is a sign of severe drought. In our experiments, the stomatal conductance never fell below 0.1 mol m^{-2} s^{-1} even though the water content had dropped to 0.14 m^3 m^{-3}, suggesting there was no severe drought stress but rather mild to moderate drought stress. This hypothesis is supported by the relative leaf water content (RWC) values. In neither year were there significant differences in RWC except at 127 DAS in 2016, where a reduction was seen after plants had not been watered for 70 days. Another canopy indicator for

297 drought stress is canopy temperature, with drought stressed plants having a higher canopy
298 temperature compared to well-watered plants (Jackson et al. 1981; González-Dugo et al. 2006; Grant
299 et al. 2006; Panigada et al. 2014). In 2017, the canopy temperature was measured for a short period
300 around the time of re-watering of the EDR plants. As soon as re-watering occurred there was an
301 immediate response in canopy temperature, even before changes in stomatal conductance or RWC
302 were seen. Therefore, the canopy temperature method could prove to be a quick and simple
303 measurement for early detection of water stress. In addition, the SPAD measurements showed an
304 increase in SPAD when drought occurred, this was most likely caused by a concentration effect.
305
306 Despite the drought in these experiments being mild to moderate, there were strong impacts on
307 water use efficiency (WUE) under prolonged drought in 2016. The DR treated plants had a WUE of
308 8.4 g ml^{-1}, compared to 6.7 g ml^{-1} in the control plants. Previous studies have shown that drought
309 results in lower WUE in C3 cereal crops and sugar beet (Araus et al. 2002; Bloch et al. 2006; Rinaldi
310 and Vonella 2006). Although it has also been seen that the WUE increases when there is drought
311 stress (Rytter 2005). In 2016 the WUE was higher in the DR treated plants indicating there was
312 drought stress. Blum (2005) pointed out that a higher WUE does not necessarily mean a higher yield
313 potential. High WUE as a result of lowered water uptake can be misleading and the drought might
314 have actually had a negative effect on yield. In 2016, water uptake was reduced severely in the DR
315 treated plants and this resulted in a decrease in storage root dry weight but the ratio between the
316 two turned out higher than that of the control plants. In 2017 there were hardly any differences
317 between the treatments but, overall, WUE was much lower than in 2016. A possible explanation for
318 this could be a difference in plant density. Previous studies in sorghum and sugar beet showed that a
319 higher plant density resulted in higher yield notwithstanding all plants had similar amounts of
320 available water (Blum 1970; Sögüt and Arioglu 2004). Since the plant density was higher in 2016, this
321 could explain why WUE was lower in 2017.
322
323 Storage root dry weight was used as an indicator for yield and continuous drought had a stronger
324 negative impact compared to short periods of drought. The DR treated plants had a significantly
325 lower root dry weight at the end of the experiment compared to the control plants. In 2017, no
326 significant differences were found between the root dry weight of the EDR and LDR treated plants.
327 Similar results were found in wheat when different germplasms were grown under full irrigation,
328 early drought, late drought and continuous drought; there were hardly any differences between
329 grain yield of early and late drought but continuous drought had a significantly lower yield than the
330 fully irrigated plants (Ginkel et al. 1998). Even though EDR and LDR did not result in significant

differences in root weight there were strong differences in leaf dry weight. Continuous drought resulted in 48% less leaf dry weight compared to the control plants. Possibly because of rapid leaf senescence of older leaves under drought.

Wetting and drying cycles can have a substantial impact on the nutrient mobilization and the rate of organic matter decomposition (Majdalani et al. 2008; Zhu and Cheng 2013). It was found that a certain amount of water can mobilize more nutrients when the irrigation is interrupted (drying) (Zhuang et al. 2007). This could have happened to some degree during the irrigated period in all treatments, where water was given several times a week for a set period. The process of slow drying between two irrigation events can strongly affect the soil aggregation and microbial activity (Zhu and Cheng 2013). This leads to changes in rhizosphere respiration, which in turn, affects the decomposition speed of the soil organic matter. This suggests that the treatments that were irrigated could have had an increased decomposition rate and therefore root death might have been more pronounced in irrigated treatments. The root images taken showed more root browning in the irrigated boxes which could have been an indication of increased decomposition rates. Van Noordwijk et al. (1994) showed that sugar beet roots have a median life span of 87 days at 10-30 cm depth. This means that the browning of roots and eventual root death is seen in field conditions, is very similar to what was found in our box experiments. At the same time there might have been differences of this effect throughout the box as a result of fixed partial root zone irrigation. Since trickle tape was used to irrigate the boxes there was an area close to the soil surface between the trickle tapes that never got replenished. Previous studies showed that the amount of micro-organisms was substantially lower in the dry soil sections of partially irrigated systems (Wang et al. 2006; Hu et al. 2011). This could be an indication for reduced root growth as well. However, it was thought that the depletion zone did not stretch to below 20 cm. There have even been studies to show that partial root zone drying can lead to improved water use efficiency (Kang and Zhang 2004).

Not much is known about the genetic variation in the rooting properties of current sugar beet varieties. Breeders might be interested to investigate the genetic variation in terms of rooting depth and where root proliferation occurs in different varieties. This could eventually be translated into recommendations depending on soil type and average weather patterns for a certain region. As well as looking at root architectural traits breeders could look for varietal differences in the rate of xylem maturation which has been found to limit water uptake for a few weeks after new roots have formed (Fitters et al. 2017).

5. Conclusion

When water availability was reduced, sugar beet responded by proliferating roots at depth. There was little root proliferation in the top 30 cm as a result of drought stress. When new roots were formed there was often a delay before water was actually taken up. It would therefore have been beneficial if roots had already been in place before drought stress occurred. Continuous drought had the most negative effects, resulting in a drastic reduction in stomatal conductance and leaf and root dry weight. Shorter periods of drought followed by re-watering showed only temporary decreases in stomatal conductance and there were no strong impacts on root dry weight or sugar yield. The differences observed in root dry weight between the years could be attributed to the difference in plant density in the experiments, with there being a higher plant density in 2016 than 2017. Future studies might look at the effects of drought stress with different intensities, timings and durations and possibly even the effects of low water availability under different plant densities.

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520 Supplementary tables

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522 Table 3 Weekly amounts of water given (mm) in both 2016 and 2017. At the start of each experiment water was given via a
523 hose and therefore no exact amounts were registered. IR = Full irrigation (control), DR = Drought from 58 DAS, EDR = Early
524 drought (60-145 DAS), and LDR = Late drought (128-178 DAS).

2016			2017		
Treatment receiving water	**Week**	**Water given (mm)**	**Treatment receiving water**	**Week**	**Water given (mm)**
IR/DR	1	na	EDR/LDR	1	na
IR/DR	2	na	EDR/LDR	2	na
IR/DR	3	na	EDR/LDR	3	na
IR/DR	4	na	EDR/LDR	4	na
IR/DR	5	na	EDR/LDR	5	36.42554
IR/DR	6	na	LDR	6	36.25386
IR/DR	7	na	LDR	7	25.91146
IR/DR	8	na	LDR	8	24.51935
IR	9	35.49383	LDR	9	12.58617
IR	10	0	LDR	10	6.882716
IR	11	77.16049	LDR	11	2.523148
IR	12	154.321	LDR	12	4.050926
IR	13	77.16049	LDR	13	4.421296
IR	14	334.8765	LDR	14	13.5108
IR	15	179.0123	LDR	15	24.33642
IR	16	435.1852	LDR	16	31.27701
IR	17	310.1852	LDR	17	37.65818
IR	18	257.716		18	0
IR	19	256.1728		19	0
				20	0
			EDR	21	33.71142
			EDR	22	36.4892
			EDR	23	26.35031
			EDR	24	86.14969
			EDR	25	108.4568
			EDR	26	96.85957
			EDR/LDR	27	12.09105
			EDR/LDR	28	17.94367
			EDR/LDR	29	25.92593
			EDR/LDR	30	19.19753
			EDR/LDR	31	15.3

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